# LARGE-VOLUME FILTRATION FOR RECOVERY AND CONCENTRATION OF *ESCHERICHIA COLI* O157:H7 FROM GROUND BEEF*

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## ABSTRACT

*Rapid assays for foodborne pathogens currently require an enrichment step to bridge the gap between the detection limit of interest (1 cfu/g or less) and the detection limits of available assay systems (100–100,000 cfu/mL). Although filtration can potentially separate and concentrate bacterial pathogens to detectable levels if large (100–1,000 mL) sample volumes are processed, prior filtration methods failed to approach these volumes because of the high solids content and viscosity of typical food samples. This report describes a novel three-stage filtration system based on a leukocyte removal filter, a glass fiber prefilter, and a membrane capture filter. Data are presented on factors (e.g., particle size, bacteria binding, pH) affecting filtration performance and protocol design.* Escherichia coli *O157:H7 at less than 1 cfu/g were quantitatively recovered from 10 g of stomached ground beef in 15 min, and detected on selective media within 24 h.*

## PRACTICAL APPLICATIONS

The methodology presented in this study allows the rapid concentration of *Escherichia coli* O157:H7 from the large volumes of stomached ground beef. The primary application is in rapid biosensor detection and quantitation of low levels of pathogens in foods without enrichment. The isolated bacteria can be recovered in a volume of ~20 μL and are virtually free from interfering

*Mention of brand or firm names does not constitute an endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

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sample constituents, allowing facile detection by polymerase chain reaction and other approaches. This filtration methodology may also be useful for studies on attachment and partitioning of bacteria on food surfaces, as free and bound bacteria can be rapidly separated at low concentrations reflecting actual contamination situations.

## INTRODUCTION

Rapid assays capable of detecting and quantitating low levels of pathogens (1–100 cfu/25 g) are important for ensuring the safety of foods that are processed and consumed quickly, such as ground beef and fresh produce. Recent advances in detection technology (Ibrahim 1986; Hadas *et al.* 1992; Feng 1996; Yu and Bruno 1996; Tu *et al.* 2001) have produced methods that can detect pathogens in minutes, but only at concentrations of ~1,000 cfu/mL or more. Detecting lower concentrations requires enrichment steps that add hours or days to the overall assay time. Enrichment also eliminates the possibility of quantitation, and does nothing to eliminate sample components that interfere with detection of the target organism. Although separation steps such as immunomagnetic bead isolation can alleviate the latter problem (Fratamico *et al.* 1992), they are limited to low sample volumes and require considerable sample manipulation.

The need for alternatives to enrichment has been discussed in a number of reviews (Payne and Kroll 1991; Sharpe 1997; Stevens and Jaykus 2004) and filtration is frequently identified as a potential strategy. If a sufficiently large sample can be processed and concentrated, filtration can allow quantitative assay results, avoid problems with the growth of nontarget microflora, remove interfering substances and provide single-step processing of samples with few manipulations. A practical filtration method would require processing 100–1,000 mL of liquid sample and concentrating a significant proportion of the target bacteria into a volume of 10–100 μL in 30–90 min. This would allow the detection of pathogens at levels approaching 1 cfu/g within 2 h using existing rapid detection methods, and represent a significant advance in rapid pathogen detection methodology.

This work describes results of experiments aimed at rational design of a filtration system using data on properties of the filter materials and samples. Filtration-based assays of homogenized foods have been used for many years (Sharpe *et al.* 1979; Pettipher and Rodrigues 1982; Tortorello and Stewart 1994), but they are limited to small volumes (~5 mL), even with the use of dilution, prefiltration and detergent and enzyme treatments (Entis *et al.* 1982; Joly *et al.* 2001). Filtration of larger volumes (100 mL) has been reported recently (Chen *et al.* 2005; Wolffs *et al.* 2006) for samples derived from

rinsing or washing foods. These results provide protocols for recovery of filter-captured organisms and demonstrate the potential of large-volume filtration to produce clean, highly concentrated samples for rapid assays. However, these methods are not applicable to typical food homogenates, which have far higher solids content. This report describes a novel three-stage filtration system based on a leukocyte removal filter, a glass fiber prefilter and a membrane capture filter. Data on properties of filters and samples are presented, as well as a protocol for rapid separation and concentration of *Escherichia coli* O157:H7 from stomached ground beef.

## MATERIALS AND METHODS

### Materials

Water was deionized in-house with a Nanopure water treatment system (Barnstead, Dubuque, IA). Woven nylon, polyester and polypropylene screen filters were from Small Parts, Inc. (Miami Lakes, FL). Membrane screen filters of native and blackened track etched polycarbonate (0.2-µm pore size) were from Poretics, Inc. (Livermore, CA). Various screen and depth filter materials including nylon stockings, vacuum cleaner bags and fabrics were obtained from the local markets and hardware stores. Purecell RCQ filters were from Pall (East Hills, NY). Swinnex holders (Millipore, Billerica, MA) were used for thicker filter materials and Swin-Lok holders (Whatman, Inc., Florham Park, NJ) were used for thinner materials. Laboratory filter paper, glass fiber filters, Triton X-100 and Tween-20 were from Sigma (St. Louis, MO). Ground beef (17% fat), ground turkey and hot dogs were obtained from the local markets. Brain–heart infusion (BHI) media was from Difco (Sparks, MD). R&F chromogenic plating medium for *E. coli* O157:H7 (RFCPM) was from R&F Laboratories (Downers Grove, IL). Flat-bottomed polystyrene microwell plates and filter holders were from Fisher Scientific (Philadelphia, PA). Prepared dry phosphate-buffered saline (PBS) and tris-buffered saline (TBS) buffers were from Pierce (Rockford, IL). All other chemicals used were of reagent grade.

### Bacterial Cultures

*Listeria monocytogenes* ATCC 19115 and *E. coli* O157:H7 ATCC 43,895 were acquired from the American Type Culture Collection (Manassas, VA). *Salmonella enterica* ssp. *enterica* serovar Typhimurium KR01 was a laboratory isolate obtained from Dr. K. Rajkowski, United States Department of Agriculture. All bacteria were cultured in BHI liquid broth or on BHI agar (BHIA) plates. Overnight cultures were prepared by inoculating 3 mL of BHI broth in a 15-mL tube with a single colony and incubating at 37C with shaking

at 250 rpm for 16–20 h. Working samples were prepared by serial 10× dilution of an overnight culture in PBS, and addition of the appropriate volume of one of these dilutions to the desired diluent. For enumerating bacteria, 200 μL of appropriate dilutions in PBS were spread on BHIA plates, incubated overnight at 37C and colonies counted manually. Enumeration was also performed with a microplate growth assay (Brewster 2003a) where indicated. For selective detection of *E. coli* O157:H7, 200 μL of liquid was spread on the surface of an RFCPM and incubated at 35C for 24 h. Dark blue or black colonies 1.5–2.5 mm in diameter were counted manually. For *in situ* enumeration, a polycarbonate filter with captured bacteria on one surface was placed on a BHIA or RFCPM plate (up to six filters per plate), and the plate was incubated and counted as mentioned earlier. Control experiments were conducted by filtering PBS with known levels of *E. coli* O157:H7 through polycarbonate filters and enumerating the captured cells as mentioned earlier.

## Apparatus

Particle size measurements were made with a SALD-201V laser light scattering instrument (Shimadzu Scientific Instruments, Columbia, MD). An EL 311s Microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) connected to a personal computer was used for the microplate growth assay. A peristaltic pump (EconoPump, Bio-Rad Laboratories, Hercules, CA) with a 3.2-mm internal diameter neoprene tubing was used to provide flow for the Purecell RCQ filters. A syringe or a Visiprep vacuum manifold (Supelco, Bellefonte, PA) equipped with a vacuum gauge and 16 leur inlets with individual valves and collection tubes was used to generate flow through 25-mm filters.

## Particle Size Distribution

Particle size distributions were determined by a gravimetric method and by a laser light scattering method. For gravimetric determinations, nylon mesh filters were loaded into 25-mm filter holders, then washed (water), dried and weighed. Using a syringe, 25 mL of stomached meat was passed through the filter with the largest mesh size. The filtrate was then loaded into a clean syringe and the process was repeated using the next largest mesh size. After the filtrate had been passed through all the filters, they were placed on a vacuum manifold and dried overnight with a slow stream of air. The individual filter holders were weighed to determine the mass for each fraction.

For the laser light scattering determinations, samples were diluted (if necessary) with water to give an absorbance reading between 0.090 and 0.120. Samples with absorbance below this value were measured without dilution. For samples containing large particles (>100 μm), several readings were taken with agitation between each reading and averaging of the results.

## Bacteria Retention

Filter materials were cut into 25-mm diameter disks and loaded into 25-mm filter holders. Polycarbonate and glass fiber filters were sterilized by autoclaving. Other filter materials as well as tubing and fittings were sterilized by rinsing with 70% ethanol for 5 min and air-dried. Approximately 10 mL of sample containing bacteria at ~$10^4$ cfu/mL was passed through the filter using the vacuum manifold to maintain a flow rate of ~5 mL/min. The RCQ filter was supplied as a sterile cartridge with attached tubing and connectors commonly used for transfusions. The filter connectors were aseptically removed and the filter was connected to the peristaltic pump with sterile barbed hose fittings. Sample containing bacteria was drawn through the filter with suction from the pump at a flow rate of ~10 mL/min and the filtrate was collected. The concentration of bacteria in the sample ($S$) and the filtrate ($F$) were determined by the microwell growth method, and percent retention was calculated as $100 \times (S - F)/S$.

## Staining to Determine Sample Composition

A simple staining procedure was developed based on histological protocols for fats and connective tissue. Oil red was prepared as a saturated solution in 60:40 isopropanol/water, aniline blue black was prepared at 0.7 mg/mL in 1% acetic acid. Samples were incubated at 45C during staining/washing. The sample was sequentially soaked in oil red (10 min), 70% ethanol (5 min), water (5 min), aniline blue black (10 min) and water (5 min). Staining of identifiable fat, muscle and connective tissues in a portion of beef steak showed that the two stains were specific for fat (red) and connective tissue (blue), respectively.

## Stomaching

Meat samples and diluent (9 mL per gram of meat) were placed in plastic stomacher bags with integral primary filters and subjected to stomaching at the "normal" speed setting. Unless otherwise noted, the mixing time was 120 s and samples were at room temperature. For temperature-controlled studies, the stomacher was preconditioned by placing a bag containing ice or warm water in contact with the paddles for 5 min before processing the sample. Sample and buffer were adjusted to the desired temperature before mixing, and held at the desired temperature during subsequent processing steps. The sample passing through the integral stomacher bag filter was termed primary filtrate. Sodium azide (0.02% final concentration) was added to samples not used for pathogen recovery to inhibit microbial growth and sample degradation. Settling rate was measured by placing primary filtrate in a 50-mL centrifuge tube and observing the position of the interface between the upper and lower layers as a function of time. Retentate weight was determined by suctioning all liquid

from the stomacher bag and weighing the remaining semisolid material. Dried particulate mass was determined by centrifuging 2 × 1.5 mL of primary filtrate in a preweighed 1.5 mL microfuge tube for 5 min at 10,000 rpm and discarding the supernate to give a total of 3 mL per tube. The tubes were dried to constant weight (6–8 h) at 95C, weighed, and the mass multiplied by the appropriate factor (e.g., 100/3 for 100 mL total sample) to give the total dried particulates mass.

### Concentration and Recovery of *E. coli* O157:H7 from Ground Beef

All operations were carried out at room temperature (~22C). Ground beef (40 g) was stomached in sterile distilled water (360 mL) for 120 s in a Fisher filter bag. The primary filtrate (~320 mL) was transferred to a sterile sample bottle. In some experiments the primary filtrate was allowed to settle and the upper or lower layer was transferred to a new sample bottle. For spiked samples, 100 µL of PBS containing ~100 cfu/mL *E. coli* O157:H7 from a diluted overnight culture was added to the bottle to give ~0.03 cfu/mL filtrate, and mixed for 30–90 min prior to filtration. A sterile filter train consisting of a pickup tube, Purecell RCQ filter, a 25 mm × 2.6 µm glass fiber filter, peristaltic pump, tubing and a 25 mm × 0.2 µm polycarbonate membrane filter was assembled. The pickup tube was placed in the sample bottle and the pump drew the sample through the RCQ and glass fiber filters under suction, and passed it through the polycarbonate filter under pressure. Flow was interrupted periodically and the polycarbonate capture filter was replaced so that filters were exposed to various sample volumes. Flow rate was measured by weighing the liquid emerging from the filter train for a fixed time period. For room temperature experiments the sample was agitated with a magnetic stirrer. For temperature controlled filtration, the sample bottle and the RCQ filter were immersed in a water bath and agitation was provided by bubbling nitrogen through the sample. In other experiments the sample was not agitated, and the pickup tube was kept within a few millimeters of the surface to minimize aspiration of settled particles. After filtration was complete, the polycarbonate filter holder was removed from the filter train and vacuum applied to the outlet to remove the remaining liquid. The filter membrane was removed from the holder and placed on a BHIA or RFCPM plate for enumeration of aerobic bacteria or *E. coli* O157:H7, respectively.

## RESULTS

### Bacteria Retention on Filter Materials

The retention behavior of three common foodborne pathogens in PBS on a variety of filter materials is shown in Table 1. It was expected that bacteria

TABLE 1.
RETENTION BEHAVIOR OF FILTER MATERIALS

| Filter material | Fraction retained on filter (%) | | |
|---|---|---|---|
| | *E. coli O157:H7* | *S. typhimurium* | *L. monocytogenes* |
| 0.45-µm glass fiber | 100 | 100 | 100 |
| 1.0-µm glass fiber | 99 | 100 | 100 |
| 1.6-µm glass fiber | 78 | –3 | – |
| 2.7-µm glass fiber (Type 4) | 89 | 92 | 98 |
| 2.6-µm glass fiber (Sigma) | 24 | 33 | 40 |
| 8-µm spectra mesh | 13 | 7 | 13 |
| 5-µm nylon mesh | 29 | 17 | 47 |
| 53-µm nylon mesh | 29 | 8 | 29 |
| 136-µm nylon mesh | 20 | 10 | 7 |
| 54-µm polyester mesh | 13 | 16 | 44 |
| 125-µm polyester mesh | 30 | 25 | 81 |
| Nylon stocking, black | 36 | 23 | 20 |
| Nylon stocking, tan | 24 | 12 | 19 |
| Nylon stocking, premium | 14 | 7 | 36 |
| Purecell RCQ | 23 | 34 | 39 |

Bacteria in PBS buffer at $10^4$ cfu/mL were passed through the filters. See text for details.

would exhibit higher binding to positively charged nylon surfaces than to polyester or glass, but this was not the case. Very high retention of bacteria was observed on several of the glass fiber filters tested, although retention was expected to be low based on the nominal pore size. This may be caused by binders or other surface coatings, and this illustrates that considerable care must be taken when selecting filter materials. With the exception of the high-retention glass fiber filters, all materials showed acceptable recovery of the pathogens from buffer and significantly lower retention when treated with bovine serum albumin or after exposure to stomached meat filtrate (data not shown). For example, retention of the pathogens on Purecell RCQ filters ranged from 23 to 39% when suspended in PBS, but was less than 5% when bacteria were suspended in stomached ground beef filtrate.

## Particle Size Distribution of the Stomached Meats

Two methods were used to determine particle size distribution, with neither being completely satisfactory. The gravimetric method was very time-consuming and prone to failure because of the plugging of the filters. Laser-light scattering was rapid, but there were difficulties with samples containing large, strongly scattering particles. These samples had to be diluted extensively to avoid multiple scattering, greatly reducing the signal for small particles.

Several readings had to be averaged with intermediate mixing because of the strong dependence of the signal on position and orientation of the large scatterers. Distributions of various stomached meats determined by the gravimetric method are shown in Fig. 1. Similar results were obtained with light scattering (data not shown). They show a very broad and relatively uniform distribution of particle sizes for all three sample types.

## Effect of the Stomaching Conditions

Ground beef was stomached under a variety of conditions to identify factors that might have a significant effect on filtration. Ground beef stomached in filter bags produced a cloudy pink liquid (primary filtrate) in the filtrate side, a large amount of fat coating the inner surfaces of the bag and filter and a pale pink mass staining positive for fat and connective tissue in the retentate side of the bag. The primary filtrate separated on standing into a translucent red upper layer with relatively few macroscopic particles and an optically dense white lower layer containing a high concentration of large particles. The nature of the primary filter, duration of stomaching, temperature of stomaching and nature of the diluent were varied, and the particle size distribution in the primary filtrate and the mass of retentate were observed.

Stomacher bags with integral filters from five manufacturers were tested. Three of the bags had full-width perforated plastic filters, one had a full-width woven mesh bag and one had a lateral nonwoven polymer filament filter of much smaller area. Particle size distributions and particle layer volumes were very similar for the large-area filter bags (data not shown). The small-area filter bag produced a much smaller percentage of large particles, but it was not possible to collect more than 40 mL of filtrate because of filter clogging. Further studies used the Whirlpak or Fisher bags with full-width perforated plastic filters.

Stomaching for 30, 60, 120 and 240 s produced no significant effect on particle size distribution (data not shown). As shown in Table 2, there was an increase in the volume of the particle layer with stomaching time, but no apparent trend in the mass of the retained solids or the filtrate particulates. Stomaching for 60 s at 4, 22 and 37C also had no discernible effect on particle size distribution or the retained mass (data not shown).

The nature of the diluent did have a substantial effect on the rate at which the lower (particle) layer of the primary filtrate separated, on the final volume of the particle layer and on filtration rate. Water was initially selected as a diluent simply to save time and expense when stomaching, filtering and characterizing large numbers of samples. Water gave primary filtrates that separated quickly to give a nearly transparent upper layer and a compact particle layer (~10% of the total volume). TBS gave filtrates that were similar to water,

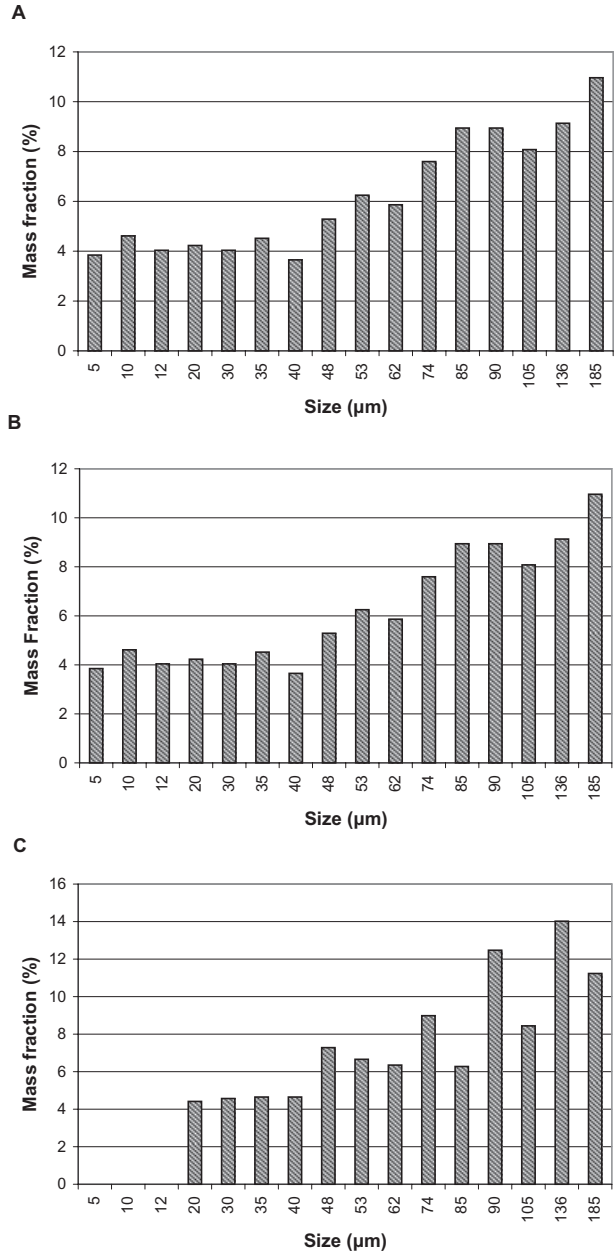


FIG. 1. GRAVIMETRIC PARTICLE SIZE DISTRIBUTION OF STOMACHED MEATS DETERMINED BY GRAVIMETRIC METHODS
(A) Hot dog. (B) Ground beef. (C) Ground chicken.

TABLE 2.
EFFECT OF STOMACHING TIME ON GROUND BEEF HOMOGENATE

| Stomaching time (s) | Particle layer volume % | Retentate mass (g) | Dried particle mass (g) |
|---|---|---|---|
| 30 | 8.0 | 9.3 | 0.41 |
| 60 | 9.5 | 8.5 | 0.43 |
| 120 | 10.0 | 8.9 | 0.54 |
| 240 | 12.5 | 9.5 | 0.45 |

10 g ground beef + 90 mL water were stomached at room temperature in Whirlpak filter bags for the indicated period.

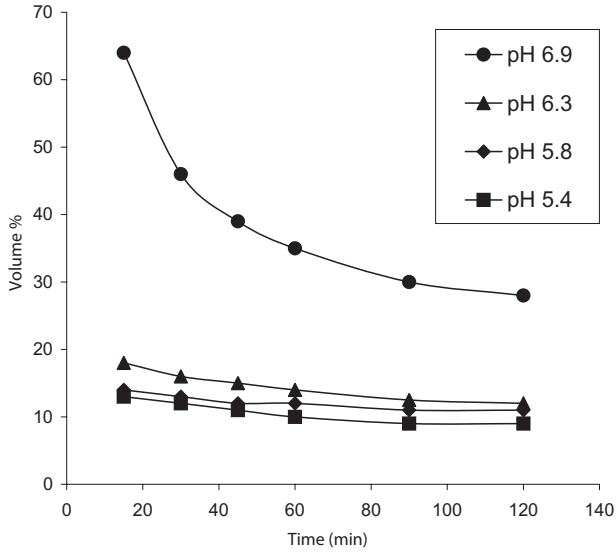


FIG. 2. EFFECT OF pH ON SETTLING OF LARGE PARTICLES IN GROUND BEEF

although settling time was longer. However, PBS filtrates separated slowly into a turbid upper layer and a diffuse particle layer (~25% of the total volume). Several experiments were conducted to determine whether the difference between PBS and water was caused by pH, ionic strength, or the presence of multivalent anions. Results are summarized in Fig. 2 and Table 3. The pH of the diluent had a strong effect on the volume of the particle layer, whereas ionic strength and multivalent anions had a modest effect. Particles from the water filtrate stained as connective tissue, whereas particles from PBS filtrates took up both the fat and connective tissue stain.

TABLE 3.
EFFECT OF DILUENT ON STOMACHED GROUND BEEF PRIMARY FILTRATE

| Diluent | Initial pH | Final pH | Particle layer volume (%) |
|---|---|---|---|
| Water | 7.0 | 5.4 | 9 |
| Water + 1 mL 1% NaOH | – | 5.8 | 11 |
| Water + 2 mL 1% NaOH | – | 6.2 | 12 |
| Water + 4 mL 1% NaOH | – | 6.8 | 28 |
| NaCl (0.3 M) | 7.0 | 6.0 | 14 |
| $NaCl/Na_3PO_4$ (0.15 M/0.1 M) | 7.2 | 7.0 | 25 |
| $NaCl/Na_4PO_4$ (0.15 M/0.1 M) | 6.0 | 6.1 | 12 |
| NaCl/Tris (0.15 M/0.025 M) | 7.2 | 6.1 | 12 |
| $NaCl/Na_2SO_4$ (0.15 M/0.1 M) | 7.0 | 5.9 | 16 |
| $NaCl/Na_2BO_4$ (0.15 M/0.1 M) | 8.5 | 8.0 | 52 |
| $NaCl/Na_2BO_4$ (0.15 M/0.1 M) | 6.3 | 6.5 | 16 |

10 g ground beef + 90 mL diluent. Phosphate-buffered saline contained 0.1 M phosphate and 0.15 M NaCl. Tris-buffered saline contained 0.025 M Tris, 0.15 M NaCl.

## Filtration of Stomached Ground Beef

Filtration of stomached ground beef primary filtrate was initially evaluated with many combinations of filter materials other than Purecell RCQ. It was found that a 2.6-µm glass fiber prefilter in series with a 0.2-µm polycarbonate filter was effective in capturing bacteria from a large volume of sample at high flow rate, provided that particles larger than ~10 µm were first removed. However, no single material or combination of two or three materials was able to remove larger particles from more than a few mL of sample before clogging (defined as a 66% reduction in flow rate from the initial value). The Purecell RCQ filter, one of a number of leukocyte removal filters available for removal of white blood cells from whole blood during transfusion (Meyer *et al.* 2005), was then tested. Large volumes of primary filtrate could be pumped through the RCQ filter, and this secondary filtrate did not clog the glass fiber/polycarbonate capture stage. The RCQ/glass fiber/polycarbonate filter combination was further characterized with ground beef stomached in a number of diluents. Representative data from different diluents and fractions from gravitational settling are shown in Fig. 3. In general, the volume filtered before clogging was very small for the lower (particle) layer, high for the upper layer, and intermediate for mixed layers. Addition of 1% Triton X-100 (Sharpe *et al.* 1979) significantly enhanced filterability, especially for mixed water homogenates. Filtration of mixed homogenates at 37C resulted in significant increase in the initial flow rate, but the filter clogged after ~120 mL (data not shown). Because large particles in ground beef stomached in water (or in buffers with final pH < 6.3) settled quickly, it was feasible to pump only the

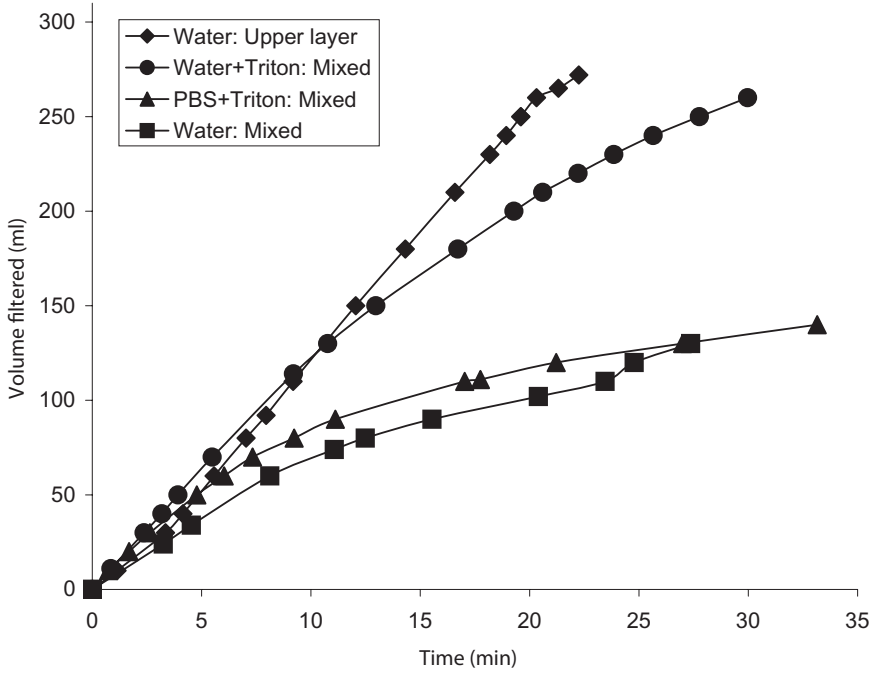


FIG. 3. FILTRATION OF GROUND BEEF STOMACHED 120 S IN VARIOUS DILUENTS
See text for details.

upper layer to the filter by keeping the pickup tube near the liquid surface. More than a liter of sample could be passed through the RCQ filter before a significant reduction in flow rate was observed using this approach.

## Recovery of *E. coli* O157:H7 from Ground Beef

Ground beef primary filtrate was spiked with ~10 cfu (~0.25 cfu/g meat, ~0.03 cfu/mL filtrate) of *E. coli* O157:H7 and pumped through a three-stage RCQ/glass fiber/polycarbonate filter. The polycarbonate capture filter was replaced periodically so that filters were exposed to 50, 100 and 230 mL of filtrate. Captured *E. coli* O157:H7 were detected by direct plating of the capture filters on selective media and produced one to three positive colonies on all filters, representing a recovery of ~50%. Negative control experiments with unspiked primary filtrate yielded no positive colonies, whereas positive controls (PBS containing 0.03–10 cfu/mL) provided the expected number of positive colonies.

## DISCUSSION

Filtration of large volumes of homogenized food samples can potentially provide very rapid detection of pathogens by eliminating the need for enrichment. The task is challenging, as typical food homogenates are complex, viscous materials with high concentrations of particles having a wide range of sizes. Conventional laboratory filter materials are only able to process a few mL of such samples before filters clog and flow rates become too low for practical use (Sharpe *et al.* 1979). This study aimed at evaluating a wide range of filter materials that might be used alone or in combination to overcome this problem. Ground beef was selected as the sample matrix because it contains high levels of fat, proteins and particulates that present a "worst case scenario" for filtration. *E. coli* O157:H7 was selected as the primary target pathogen because it is closely associated with foodborne disease in ground beef. Plating on selective media was used for detection because it provided a simple and unequivocal means to demonstrate concentration and recovery of a target pathogen in the presence of large numbers of background microorganisms.

After evaluating a wide range of filter materials and investigating the properties of ground beef homogenates produced by stomaching under a variety of conditions, a protocol for the recovery of *E. coli* O157:H7 from ground beef at levels below 1 cfu/g was established. A novel three-stage filtration system (leukocyte removal filter, glass fiber prefilter, polycarbonate membrane capture filter) provided a sample flow rate of 10 mL/min and a sample capacity of at least 100 mL. Sample preparation time, including weighing and stomaching the sample, was less than 45 min. It was found that pH during stomaching had a significant effect on the properties of stomached ground beef – by keeping pH below 6.3, large particles in the primary filtrate settled quickly and more than 1,000 mL of the upper layer could be filtered at high flow rates. It should be noted that the use of water as a diluent could lead to osmotic injury or death of bacteria, and sample osmolarity should be maintained at appropriate levels to prevent this. In this work the addition of unstressed pathogen after the sample was homogenized and released salts and proteins avoided significant osmotic injury.

Plate counting was used for detection here, but established methods for quickly recovering whole cells (Chen *et al.* 2005) and DNA (Alvarez *et al.* 1994; Bej *et al.* 1991; Wolffs *et al.* 2006) from filters would allow large volume filtration to be coupled to many existing pathogen detection systems. This would provide sensitive, quantitative assays with much shorter assay times than methods using enrichment. This filtration methodology may also be useful for studies on attachment and partitioning of bacteria in foods. The first two stages of the filter system may be used with bacteria separation methods such as immunoaffinity columns (Brewster 2003b) or with filter-based

detection platforms (Bernal *et al.* 1994; Brewster and Mazenko 1998). Work remains to be done to further characterize filter materials and food samples, determine optimum conditions for release of pathogens from food particles, optimize filtration protocols and integrate filtration with rapid detection methods. This study represents the initial step in this direction, extending existing data on filter materials and demonstrating that filtration is a viable approach for recovery and concentration of low levels of pathogens from concentrated food homogenates.

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